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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PCT/ST-22		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IT98/00364	16/12/1998	19/12/1997
International Patent Classification (IPC) or national classification and IPC C07K14/47		
Applicant SIGMA-TAU INDUSTRIE FARMACEUTICHE RIUNITE...et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which hav been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14/07/1999	21.03.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Armandola, E Telephone No. +49 89 2399 7493

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IT98/00364

I. Basis of the r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-11	as originally filed	
12	with telefax of	04/02/2000

Claims, No.:

1-12	with telefax of	04/02/2000

Drawings, sheets:

1/6-4/6	as originally filed	
5/6,6/6	with telefax of	04/02/2000

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. (4, 9) partially (N, IS, IA); 6-8 (IA).

because:

☒ the said international application, or the said claims Nos. 6-8 (IA) relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. (4, 9) partially are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☒ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-12
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-5, 9-12
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Novelty, Inventive step and Industrial applicability

The part of Claims 4 and 9 referring to inflammatory diseases was not examined due to the lack of disclosure regarding this part of the claims (see Item VIII, 1) ii) below).

Industrial applicability

Claims 6-8 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Art. 34(4)(a)(i) PCT).

For the assessment of the present Claims 6-8, with regard to methods of treatment of the human body, on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claim. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item IV

Lack of unity of invention

The objection to lack of unity raised by the ISA is not maintained by this authority. Therefore, all parts of the present application were subjected to International Preliminary Examination (IPE).

Re Item V

Reasoned statem nt under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document:

D1: VOURET-CRAVIARI V ET AL: 'Expression of a long pentraxin, PTX3, by monocytes exposed to the mycobacterial cell wall component lipoarabinomannan.' INFECTION AND IMMUNITY, (1997 APR) 65 (4) 1345-50. , XP002113122

Prior art

Document D1 studies the expression of mouse PTX3 upon stimulation with mycobacterial components. It suggests a role for PTX3 in mycobacterial infections but does not disclose the possible use of PTX3 in therapy of microbial infections.

Novelty and Inventive step (Art. 33(3)(4) PCT)

The subject matter of claims 1-12 can be considered novel and inventive because neither pharmaceutical compositions, nor methods of gene therapy for the treatment of tumor conditions involving the use of PTX3 nor the use of the cDNA corresponding to PTX3 in the preparation of medicaments are disclosed or suggested in the prior art.
D3 discloses that IL-1, TNF, lipopolysaccharide and mycobacterial cell wall components induce the expression and production of the long pentraxin PTX3, but does not suggest that long pentraxin PTX3 can have a therapeutic effect on infectious diseases, or on tumor conditions.
Although the claims are considered novel and inventive, it should be noted that for the subject-matter of claims 4-12 it seems that the invention is not disclosed in a manner sufficiently clear and complete to be carried out by the person skilled in the art (Art. 5 PCT) and that is not fully supported by the description (Art. 6 PCT) (see Item VIII).

Re Item VIII
Certain observations on the international application

Sufficiency of disclosure and support by the description (Art.5, Art. 6 PCT)

i) With regard to Claims 4, 6-10 and 12, in reference to the use of PTX3 in the treatment of tumors, the claims are not considered to be sufficiently disclosed nor supported by the

description.

Claim 4 refers to a composition containing the PTX3 protein for the treatment of tumors: such a composition is not disclosed in the description. Instead a composition containing PTX3, useful for the recruitment of leukocytes is disclosed (see p.8). The fact that such a composition can induce leukocyte recruitment is far from indicating its usefulness in treating tumor conditions, and the skilled person would find no indication in the description that might imply so. The same holds true for Claims 9 and 10, referring to the use of the PTX3 protein for preparing a medicament for treating tumor disease and for Claim 12 referring to the use of the PTX3 DNA for the same purpose.

Claim 6 concerns a method for the treatment of an established tumor. On page 9 of the description, one example of the possibility to prevent *in vivo* proliferation of a murine mastocytoma by using PTX3 is given. In the experiment healthy mice are injected with P815 tumor cells transfected with PTX3 cDNA and tumor rejection is observed. This experiment cannot be seen as a basis for the method described in Claim 6.

The same is true for Claims 7 and 8 in which a method of gene therapy using a retroviral vector for the treatment of tumors is claimed. Such method is not disclosed in the description and is not supported by any example.

ii) Claims 4, 5 and 9-11 refer to the treatment of infectious diseases caused by bacteria, fungi, protozoa or viruses.

The prior art (D1) teaches that PTX3 is induced on monocytes by mycobacterial cell wall component and LPS; D1 suggests a possible use of long PTX3 as a diagnostic agent for infections, it does not teach or even suggests that long PTX3 can have a therapeutic effect on infectious diseases.

The application does not prove that PTX3 could be useful for the therapy of infectious diseases, and the only example that might support this notion is the leukocyte recruitment data obtained by injection of PTX3.

It is, however, well known that resistance or susceptibility to a certain microorganism, as well as the ability to successfully fight an infection rather than developing a chronic form of the disease, are linked to the quality of the immune response developed by the organism (e.g. Th1 vs. Th2 induction). The fact that PTX3 induces leukocyte recruitment does not imply that it can act as a therapeutic agent for infectious diseases, as this may depend on the type of cytokines released by the recruited leukocytes upon contact with the microorganism.

iii) In Claims 4, 9 and 10 reference is made to inflammatory diseases as targets of treatment with PTX3.
As no proof that might support the usefulness of PTX3 in treating inflammatory diseases can be found in the description, the notion that PTX3 might have a role in inflammatory diseases is of purely speculative nature (see also Item III).

INTERNATIONAL SEARCH REPORT

[redacted]tional Application No
PCT/IT 98/00364

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/47 C12N15/12 A61K38/17 C12N15/10 C12P21/02
A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOTTAZZI, B. (1) ET AL: "Preliminary biochemical and biological characterization of ptx3, a new member of the pentraxin gene family." CYTOKINE, (NOV., 1997) VOL. 9, NO. 11, PP. 903. MEETING INFO.: FIFTH ANNUAL CONFERENCE OF THE INTERNATIONAL CYTOKINE SOCIETY LAKE TAHOE, NEVADA, USA NOVEMBER 9-13, 1997 INTERNATIONAL CYTOKINE SOCIETY. , XP002113120 the whole document ---	1-5, 12-18
X	INTRONA M ET AL: "Cloning of mouse ptx3, a new member of the pentraxin gene family expressed at extrahepatic sites." BLOOD, (1996 MAR 1) 87 (5) 1862-72. , XP002113121 the whole document ---	6-11
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 1999	03/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 21313 A (ITALFARMACO) 28 October 1993 (1993-10-28)	6-11
A	the whole document	1-5, 12-18
A	VOURET-CRAVIARI V ET AL: "Expression of a long pentraxin, PTX3, by monocytes exposed to the mycobacterial cell wall component lipoarabinomannan." INFECTION AND IMMUNITY, (1997 APR) 65 (4) 1345-50. , XP002113122 the whole document	1-18

PATENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 19 October 1999 (19.10.99)	
International application No. PCT/IT98/00364	**Applicant's or agent's file reference** PCT/ST-22
International filing date (day/month/year) 16 December 1998 (16.12.98)	**Priority date** (day/month/year) 19 December 1997 (19.12.97)
Applicant BOTTAZZI, Barbara et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

14 July 1999 (14.07.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Maria Victoria CORTIELLO

Telephone No.: (41-22) 338.83.38

Brief description of drawings

Figur 2: PTX3 binding to C1q. Panel A shows the binding of the supernatant of the culture containing PTX3 (sense) and of the purified protein to C1q and C1s immobilised on plate. The binding is assessed as optical density (O.D.) at 405 nm. Panel B shows the saturation curve obtained with the biotinylated protein. The kinetic parameters were calculated using the non-linear fitting statistical method.

Figure 3: PTX3-induced leukocyte recruitment: 1 μg of PTX3 is injected into a subcutaneous pocket induced in the back of CD1 mice by inoculation of 5 ml of air.

Figure 4: PTX3-induced leukocyte recruitment in normal animals and in genetically modified animals without C1q. PTX3 is injected into a subcutaneous induced on the back of the animals.

Sequence 1: Amino acid sequence of human PTX3. The underlined amino acids constitute the peptide signal. Mature hPTX3 consists of 364 amino acids.

Sequence 2: Nucleotide sequence of fragment of cDNA of human PTX3. Upper case letters denote nucleotides coding for the protein, while lower case letters denote regions at 3' and 5' not translated but present in the construct.

CLAIMS

1. Orally, parenterally, transdermally or subcutaneously administrable pharmaceutical composition containing as active ingredient the amino acid sequence of the long pentraxin PTX3, and a pharmacologically acceptable excipient.
2. Composition according to claim 1, in which the sequence of the long pentraxin PTX3 is the sequence of naturally occurring PTX3.
3. Composition according to claim 2, in which the sequence of the long pentraxin PTX3 is the sequence of human PTX3.
4. Composition according to claims 1-3, for the treatment of infectious and inflammatory diseases or tumours.
5. Composition according to claim 4, for the treatment of diseases caused by bacteria, fungi, protozoa o viruses.
6. Expression vector containing the complete cDNA sequence coding for the long pentraxin PTX3 or one of its functional derivatives.
7. Vector according to claim 6, which is a plasmid.
8. Vector according to claim 7, which is the plasmid pSG5.
9. Recombinant host cells transfected with the expression vector of claims 6-8.
10. Host cells according to claim 9, which are CHO cells.
11. Method for producing long pentraxin PXT3 or one of its functional derivatives comprising the culturing of the cells of claims 9-10.

12. Method of gene therapy for the treatment of tumour conditions, comprising:

(a) taking samples of cells from a patient suffering from a cancer;

(b) transfecting such cells with an expression vector containing the complete cDNA sequence coding for the long pentraxin PTX3 or one of its functional derivatives; and

(c) inoculating said patient with such transfected cells.

13. Method of gene therapy for the treatment of tumour conditions comprising:

a) preparation of an expression vector of viral origin containing the complete cDNA sequence coding for the long pentraxin PXT3 or one of its functional derivatives; and

b) injection of the expression vector thus obtained into a patient suffering from a cancer.

14. Method according to claim 13, in which the expression vector of viral origin is an adenovirus or retrovirus.

15. Use of the long pentraxin PTX3 for the preparation of a medicament for the treatment of infectious, inflammatory or tumour diseases.

16. Use according to claim 15, in which the long pentraxin PTX3 is the human pentraxin having sequence 1.

17. Use according to claim 16, for the preparation of a medicament for the treatment of diseases caused by bacteria, fungi, protozoa or viruses.

18. Use of cDNA coding for the long pentraxin PTX3 or one of its functional derivatives for the preparation of expression vectors containing such cDNA to be used in gene therapy methods for the treatment of tumour conditions.

Sequence 1

			M	1
HLLAILFCAL	WSAVLAENSD	DYDLMYVNLD	NEIDNGLHPT	41
EDPTPCDCGQ	EHSEWDKLFI	MLENSQMRER	MLLQATDDVL	81
RGELQRLREE	LGRLAESLAR	PCAPGAPAEA	RLTSALDELL	121
QATRDAGRRL	ARMEGAEAQR	PEEAGRALAA	VLEELRQTRA	161
DLHAVQGWAA	RSWLPAGCET	AILFPMRSKK	IFGSVHPVRP	201
MRLESFSACI	WVKATDVLNK	TILFSYGTKR	NPYEIQLYLS	241
YQSIVFVVGG	EENKLVAEAM	VSLGRWTHLC	GTWNSEEGLT	281
SLWVNGELAA	TTVEMATGHI	VPEGGILQIG	QEKNGCCVGG	321
GFDETLAFSG	RLTGFNIWDS	VLSNEEIRET	GGAESCHIRG	361
NIVGWGVTEI	QPHGGAQYVS			381

Sequence 2

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        ctca aactcagctc acttgagagt ctcctcccgc cagctgtgga aagaactttg      54
cgtctctcca gcaATGCATC TCCTTGCGAT TCTGTTTTGT GCTCTCTGGT CTGCAGTGTT      114
GGCCGAGAAC TCGGATGATT ATGATCTCAT GTATGTGAAT TTGGACAACG AAATAGACAA      174
TGGACTCCAT CCCACTGAGG ACCCCACGCC GTGCGACTGC GGTCAGGAGC ACTCGGAATG      234
GGACAAGCTC TTCATCATGC TGGAGAACTC GCAGATGAGA GAGCGCATGC TGCTGCAAGC      294
CACGGACGAC GTCCTGCGGG GCGAGCTGCA GAGGCTGCGG GAGGAGCTGG GCCGGCTCGC      354
GGAAAGCCTG GCGAGGCCGT GCGCGCCGGG GGCTCCCGCA GAGGCCAGGC TGACCAGTGC      414
TCTGGACGAG CTGCTGCAGG CGACCCGCGA CGCGGGCCGC AGGCTGGCGC GTATGGAGGG      474
CGCGGAGGCG CAGCGCCCAG AGGAGGCGGG GCGCGCCCTG GCCGCGGTGC TAGAGGAGCT      534
GCGGCAGACG CGAGCCGACC TGCACGCGGT GCAGGGCTGG GCTGCCCGGA GCTGGCTGCC      594
GGCAGGTTGT GAAACAGCTA TTTTATTCCC AATGCGTTCC AAGAAGATTT TTGGAAGCGT      654
GCATCCAGTG AGACCAATGA GGCTTGAGTC TTTTAGTGCC TGCATTTGGG TCAAAGCCAC      714
AGATGTATTA AACAAAACCA TCCTGTTTTC CTATGGCACA AAGAGGAATC CATATGAAAT      774
CCAGCTGTAT CTCAGCTACC AATCCATAGT GTTTGTGGTG GGTGGAGAGG AGAACAAACT      834
GGTTGCTGAA GCCATGGTTT CCCTGGGAAG GTGGACCCAC CTGTGCGGCA CCTGGAATTC      894
AGAGGAAGGG CTCACATCCT TGTGGGTAAA TGGTGAACTG GCGGCTACCA CTGTTGAGAT      954
GGCCACAGGT CACATTGTTC CTGAGGGAGG AATCCTGCAG ATTGGCCAAG AAAAGAATGG      1014
CTGCTGTGTG GGTGGTGGCT TTGATGAAAC ATTAGCCTTC TCTGGGAGAC TCACAGGCTT      1074
CAATATCTGG GATAGTGTTC TTAGCAATGA AGAGATAAGA GAGACCGGAG GAGCAGAGTC      1134
TTGTCACATC CGGGGGAATA TTGTTGGGTG GGGAGTCACA GAGATCCAGC CACATGGAGG      1194
AGCTCAGTAT GTTTCAtaaa tgttgtgaaa ctccacttga agccaaagaaa gaaactcac      1254
acttaaaaca catgccagtt gggaaggtct gaaaactcag tgcataatag gaacacttga      1314
gactaatgaa agagagagtt gagaccaatc tttatttgta ctggccaaat actgaataaa      1374
cagttgaagg aaagacattg gaaaaagctt                                      1404
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